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Evaluation of the effect of different erosive drinks on teeth - Saudi Arabia, 2020

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ABSTRACT

Aim: The purpose of this in-vitro study was to investigate the effect of different erosive drinks on the microhardness of tooth enamel. *Materials and methods*: This in-vitro investigation utilized 48 extracted premolar teeth collected from the orthodontic clinics. Teeth crowns were ground 2mm on vestibular surfaces by ISOMET 2000 precision saw and cleaned in an ultrasonic bath. Teeth were divided into four groups based on immersion in different erosive drinks (Pepsi, Red Bull, Lemon, and Artificial Saliva), containing 12 teeth. Teeth were immediately kept in artificial saliva incubated at 37° for one day to simulate the oral environment. Later, pH values and surface micro-hardness were recorded using Buehler Micromet II and Vickers hardness methods. Descriptive statistics and ANOVA tests were applied to the data. *Results*: The pH values of Lemon drink (4.44±0.87), Red Bull (5.18±0.88), Pepsi (4.50±0.88), and Artificial saliva (7.50±0.87) were found, respectively. Comparison of mean pH values showed a statistically significant difference by ANOVA test (F=32.62, p<0.001). Further analysis indicated that the artificial saliva had a significantly higher pH value than all other drinks. The micro hardness values of enamel immersed in Lemon drink (281.00±89.73), Red Bull (230.83±73.76), Pepsi (311.50±106.23), and Artificial saliva (277.17±92.11) were observed, respectively. Comparison of micro hardness did not show any significant difference (F=1.599, p=0.203). *Conclusion*: Acidic drinks tested in this study had pH values sufficiently low to cause erosion and softening of the dental enamel surface. The microhardness value of enamel immersed in Red Bull was lowest, suggesting higher enamel loss.

Keywords: Erosive drinks, enamel, microhardness, pH

1. INTRODUCTION

Dental wear is recognized as a significant problem in all age groups. There are three types of dental wear known for many years: erosion, attrition, and abrasion, but the contribution of erosion to dental wear isrising in recent years (Tencate and Imfeld, 1996; Corica and Caprioglio, 2014; Jaeggi and Lussi, 2014; Kitasako et al., 2015; Salas et al., 2015). Dental erosion caused by non-bacterial

acids is under-saturated for hydroxyapatite and fluorapatite, dissolving apatite crystals (Meurman and Ten Gate, 1996; Amaechi et al., 1999; Larsen and Nyvad, 1999). Erosive acids may have an intrinsic or extrinsic origin (Ganss et al., 2012). Intrinsic due to hydrochloric acid from gastric juices that reach the oral cavity through vomiting or regurgitation, while extrinsic includes certain drinks and foods (Li et al., 2012; Mulic et al., 2012; Hermont et al., 2014).

Recent studies have shown a relationship between the development of dental erosion and the intake of soft drinks, and dental erosion is positively linked to increased consumption of soft drinks and fruit drinks (Millward et al., 1994). Intake of low-calorie cola has shown less erosive potential than regular cola (Rios et al., 2009). Excess intake of smoothies and fruit juices is also harmful due to thesugar and acid-rich content. On the other hand, demineralization may occur as a direct result of consumption, leading to dental erosion and dental caries (Liska et al., 2019). The erosive possibility of soft drinks depends on many factors, including pH and buffering capacity, type of acid, frequency of exposure, duration of every erosive exposure, chelating properties, and calcium and phosphate content (Larsen, 1975). Saliva-produced pellicles provide a natural protective coating for human enamel in the oral cavity, shielding exposed tooth surfaces from both aggressive abrasive forces and dissolution caused by an excessive dietary acid attack (Hannig and Joiner, 2006). However, the published reports provided minimal data on routinely consumed erosive drinks with varying pH levels on the enamel's microhardness. Hence this in-vitro study aimed to evaluate the effect of Lemon, Red Bull, Pepsi, and Artificial saliva on the microhardness of the enamel under varying pH levels.

2. MATERIALS AND METHOD

It was an in-vitro experimental study carried out on 48 caries-free recently extracted premolars for orthodontic reasons. Ethical clearance was obtained from the research center of Riyadh Elm University (RC/IRB/2018/1511), Riyadh, Saudi Arabia. This study was conducted and finalized from October-2019 to February 2020. A sample size of 48 teeth was calculated based on the effect size of 5, the alpha error probability of 0.05, power of the study 0.080 by considering four groups each containing 12 teeth.

Teeth preparation, grouping, and Storage

Periodontal tissue remains removed, and the teeth were cleaned by using a rotating brush and fine pumice. ISOMET 2000 precision saw cutting specifications of 0.1 mm-20mm with an internal coolant and lubricant circulation facility was utilized to prepare the teeth (Figure 1), followed by cleaning in an ultrasonic bath equipped with a timer having vibration of up to 28 kHz (Figure 2). Teeth were prepared to expose the enamel's facial surface, and crowns were ground 2mm on the vestibular surface. Teeth with the vestibular surfaces facing up were horizontally embedded in polyester resin (Figure 3). All the teeth were keptin artificial saliva solutions (pH ~8.16) followed by an Incubator at 37 degrees temperature for one day to mimic the oral environmental condition.

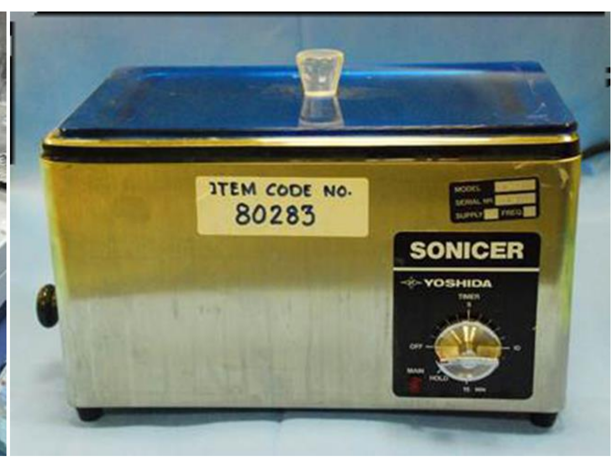

Figure 1 ISOMET Precisionsaw **Figure 2** Ultrasonic bath

Analysis of the erosive drinks

Four commonly consumed drinks (Lemon drink®, Pepsi®, Redbull®, and artificial saliva) in Saudi Arabia were chosen for the study purpose. Teeth were divided into four groups, each containing a sample of 12 teeth, and immersed in erosive drinks of varying pH.

Figure 3 Teeth embedded in resin

Figure 4 Buehler Micromet II

Measurements of enamel microhardness

After one-day immersion of the teeth in respective drinks, microhardness was measured using the Buehler Micromet II microhardness tester (Buehler, Dusseldorf, Germany) (Figure 4). Vickers hardness was evaluated by making indentations on enamel, using a pyramid diamond indenter tip exerting 100-grams load for 15 seconds. The length of the indentations on each tooth was determined using a micrometer placed on a microscope connected to the Hardness Tester. Each specimen was weighed three times. The Vickers hardness number was calculated from the standard reference table given by the manufacturer. The findings for the three hardness numbers were expressed as mean and standard deviation.

Statistical analysis

Descriptive statistics of mean and standard deviation values were calculated for the pH and enamel microhardness after immersion in various drinks. Data showed normal distribution for the microhardness values across different groups. Analysis of Variance (ANOVA) tests was applied to compare the drinks' mean pH and enamel microhardness values among different groups. Further pairwise compassion between groups was performed using Tukey's multiple comparison tests. A value of $p<0.05$ was considered significant for all statistical analyses. All the data analysis was performed using Statistical Package for Social Sciences (SPSS 25, Armonk, NY: USA)

3. RESULTS

An overall pH value of study drinks was found to be 5.41±1.51. The pH values of Lemon drink (4.44±0.87), Red Bull (5.18±0.88), Pepsi (4.50±0.88), and Artificial saliva (7.50±0.87) are displayed in Figure 5. Comparison of mean pH values in different drinks groups showed a statistically significant difference by ANOVA test (F=32.62, $p<0.001$). Further analysis indicated that the artificial showed a significantly higher pH value compared to all other drinks. However, there were no significant differences in the mean pH values between Lemon and Red Bull, Lemon and Pepsi, and Red Bull and Pepsi ($p>0.05$) were found (Table 1).

Table 1 Comparison of mean pH values among different drinks by ANOVA

Drinks	N	Mean±SD	Std. Error	F	p
Lemon	12	4.44 ± 0.87^{B}	0.25	32.62	<0.001
Red Bull	12	5.18 ± 0.88^{B}	0.25		
Pepsi	12	4.50 ± 0.88^{B}	0.25		
Artificial saliva	12	7.50 ± 0.87^{A}	0.25		

Superscript on mean values with different letters indicates a statistical significance, SD=standard deviation

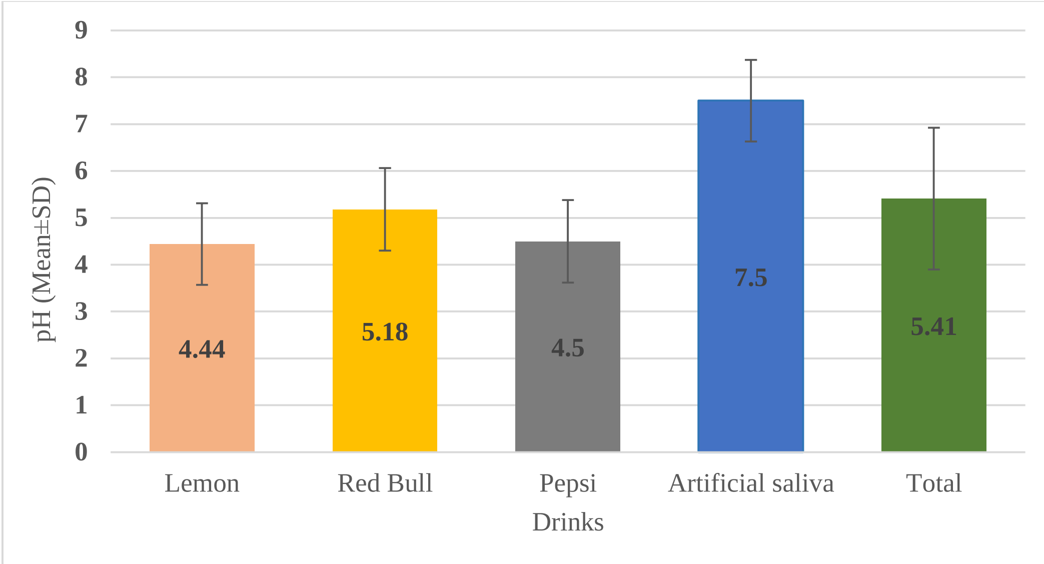

Figure 5 pH (Mean±SD) values of drinks

An overall enamel microhardness of 275.13±92.91 was observed for all the study samples immersed in various drinks. The microhardness values of enamel immersed in Lemon drink (281.00±89.73), Red Bull (230.83±73.76), Pepsi (311.50±106.23), and Artificial saliva (277.17±92.11) are displayed in Figure 6. Comparison of microhardness of teeth immersed in different drinks groups did not show any statistically significant difference by ANOVA test (F=1.599, p=0.203) (Table 2).

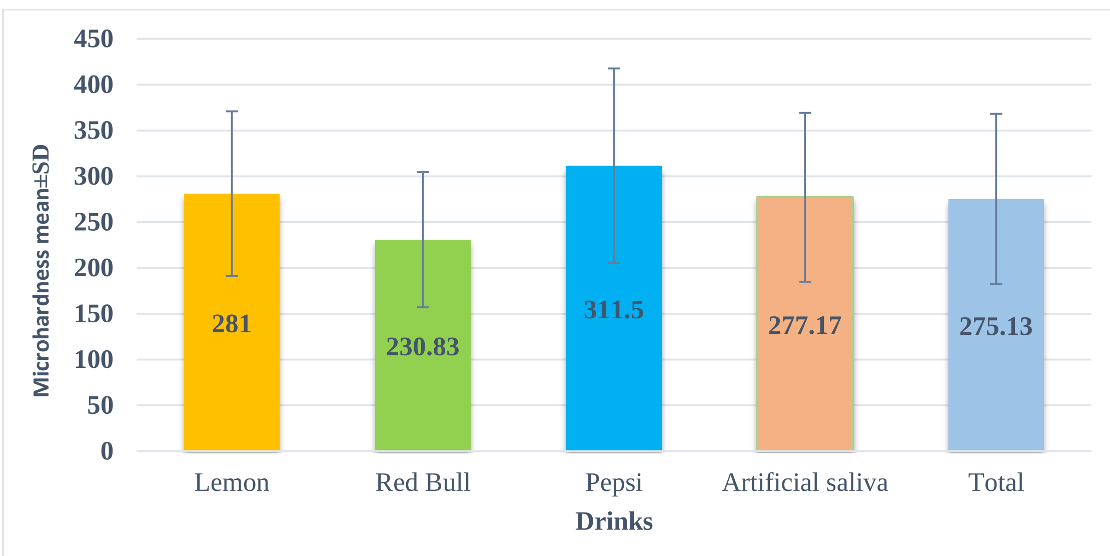

Figure 6 Enamel microhardness (VHN) values of teeth immersed in different drinks

Table 2 Comparison of mean enamel microhardness of teeth immersed in different drinks by ANOVA

Drinks	N	Mean±SD	Std. Error	F	p
Lemon	12	281.00±89.73	25.90	1.599	0.203
Red Bull	12	230.83±73.76	21.29		
Pepsi	12	311.50±106.23	30.67		
Artificial saliva	12	277.17±92.11	26.59		

Superscript on mean values with different letters indicates a statistical significance, SD=standard deviation

4. DISCUSSION

The critical pH of 5.5 is the point at which enamel dissolution occurs. Studies have indicated that several commercially marketed drinks could potentially erode enamel due to their low pH values. Previous studies have shown that the pH of carbonated soft drinks ranged between 2.3 to 3.4, and acidic fruit juices ranged from 2.1-3.6 (Seow and Thong, 2005). However, in this study, pH values ranged from 4.4 to 5.18 among carbonated drinks. The lowest pH was observed with Lemon drinks, and the highest pH was found with Red Bull. At the same time, the artificial saliva used as a control showed a pH value of 7.5. The etching effects of drinks on the enamel surface are detected within five minutes of contact and are more evident after 30 minutes. Most acidic products have the most significant impact on enamel (Grenby et al., 1990; Grenby et al., 1990). However, acid titrability and buffering capacity of the drinks have been found to impact teeth erosion. Currently, available data support that drinks directly reflect erosive capacity on tooth enamel (Larsen and Richards, 2002). In this study, teeth were immersed in the respective drinks for 24 hours, after which samples were subjected to testing.

Different physical methods like microhardness measures, enamel profile analysis, and chemical examination may be used to assess reduced enamel hardness and the mineral loss following erosion of the enamel surface by the acidic drinks (Arends and Ten Cate, 1981; Collys et al., 1993; Attin et al., 2000). In this study, the effect of the acidic drinks on the tooth enamel was tested by measuring the enamel's microhardness. The present study findings clearly show that the enamel's microhardness reduced after 24 hours of immersion in various drinks. The lowest enamel microhardness value was found with the Red Bull, followed by artificial saliva, lemon drink, and Pepsi. However, no significant difference in the mean microhardness of enamel was observed. This finding is similar to the other reported studies in which Red Bull demonstrated a rapid reduction in enamel surface hardness without any significant difference from other energy drinks (Jeong et al., 2014; Oh and Lee, 2015).

On the contrary, a significant reduction in the microhardness of dental enamel by 16 and 24 percent after treatment by Lucozade and Coca-colaand 53 percent after treatment with lime juice concentrate has been reported in the literature (Seow and Thong, 2005). The observed difference in microhardness in previous studies could be due to the enamel blocks with different properties due to bovine or rat enamel (Rytömaa et al., 1988; Sorvari, 1989). Hence the previous findings were less applicable due to species variations in enamel structure (Seow and Thong, 2005). However, in a few studies, human dental enamel blocks were utilized by removing the enamel surface and allowing subsurface enamel to react and erode quickly (Lussi et al., 2000; Eisenburger et al., 2001). In the present study, the teeth' vestibular surfaces were ground and sterilized in formalin, which might have caused the enamel surface of all the teeth to be somewhat susceptible to erosion.

Of further interest are our results, which showed that even after having a lower pH value, enamel immersed in Pepsi drink demonstrated the highest microhardness. In comparison, enamel in Red Bull showed the lowest microhardness. The underlying concept of making artificial saliva is identical to natural saliva in physical and chemical composition. The pH of artificial saliva is very different, as it varies from 5.0 to 7.3, which is similar to its physiological lower and upper limits. Applied pH depends on the dental experiment used (J Pytko-Polonczyk et al., 2017). In this study, artificial saliva having a pH of ~8.16 was utilized. Teeth immersed in artificial saliva demonstrated a mean microhardness value of 277.17±92.11 VHN. This value is higher than Red Bull immersed teeth and lower than those immersed in Lemon and Pepsi drinks.

The study's clinical applications are that persons with decreased saliva appear to have higher dental erosion than usual salivary flow (Imfeld, 1996). However, higher dental erosion occurs in athletes, leading to the regular consumption of carbonated drinks during athletic events (Khan et al., 2001). Red Bull is one of the drinks commonly consumed by athletes making teeth more susceptible to dental erosion. The study's limitations include testing of only a few commercially available drinks and a small sample size. Moreover, the present study excludes the analysis of different salivary buffer systems that may successfully neutralize acidic beverages. Future research should examine the nature of saliva buffering on acidic beverages.

5. CONCLUSION

Acidic drinks tested in this study hadpH values sufficiently lower than artificial saliva to cause erosion and softening of the dental enamel surface. There was no significant difference observed in the microhardness value of enamel immersed in various acidic beverages, although the Red Bull showed the lowest microhardness value indicating higher enamel loss. Acidic beverages are an important contributing factor in dental erosion. Therefore, the general public should be made aware of the consequence of consumption of acidic drinks on teeth

Contribution

Haya AlKanhal: conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, final approval

Mohammed AlDaij: Acquisition of data, drafting the article, final approval

Norah AlKanhal: Acquisition of data, revising the paper, final approval

MeteabAlMoither: Interpretation of data, revising the article, final approval

May Albusair: Acquisition of data, analysis and interpretation of data

Baseer MA: Analysis and interpretation of data, drafting the article, final approval

Ethical approval

The study was approved by the research center of Riyadh Elm University (RC/IRB/2018/1511).

Conflicts of interest

The authors declare that they have no conflict of interest.

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Data and materials availability

All data associated with this study are present in the paper.

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